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Rate of Gametophyte Maturation in Sexual and Apogamous Forms of Pellaea glabella

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Wagner et al (1965) have reported the occurrence of a diploid sexual form of *Pellaea glabella* var. *glabella* in Missouri. Previously, this variety was known only to be obligately apogamous at the $4x$ chromosome level (Tryon and Britton, 1958). A comparison of the haploid and tetraploid gametophytes of the sexual and apogamous forms respectively is of interest because artificially produced polyploid series of gametophytes in other fern genera (Manton, 1950; Whittier, 1966) demonstrate a number of quantitative variations. The sexual and asexual methods of sporophyte formation have different requirements, e.g., water for sperm movement, which is required for sexual reproduction, is unnecessary for apogamy. Modifications in gametophyte growth and development possibly accompany the method of sporophyte formation. The gametophytes of the sexual and apogamous forms of *P. glabella* var. *glabella* provide an opportunity to investigate prothalli of the same species with variations in chromosome number and mode of sporophyte formation.

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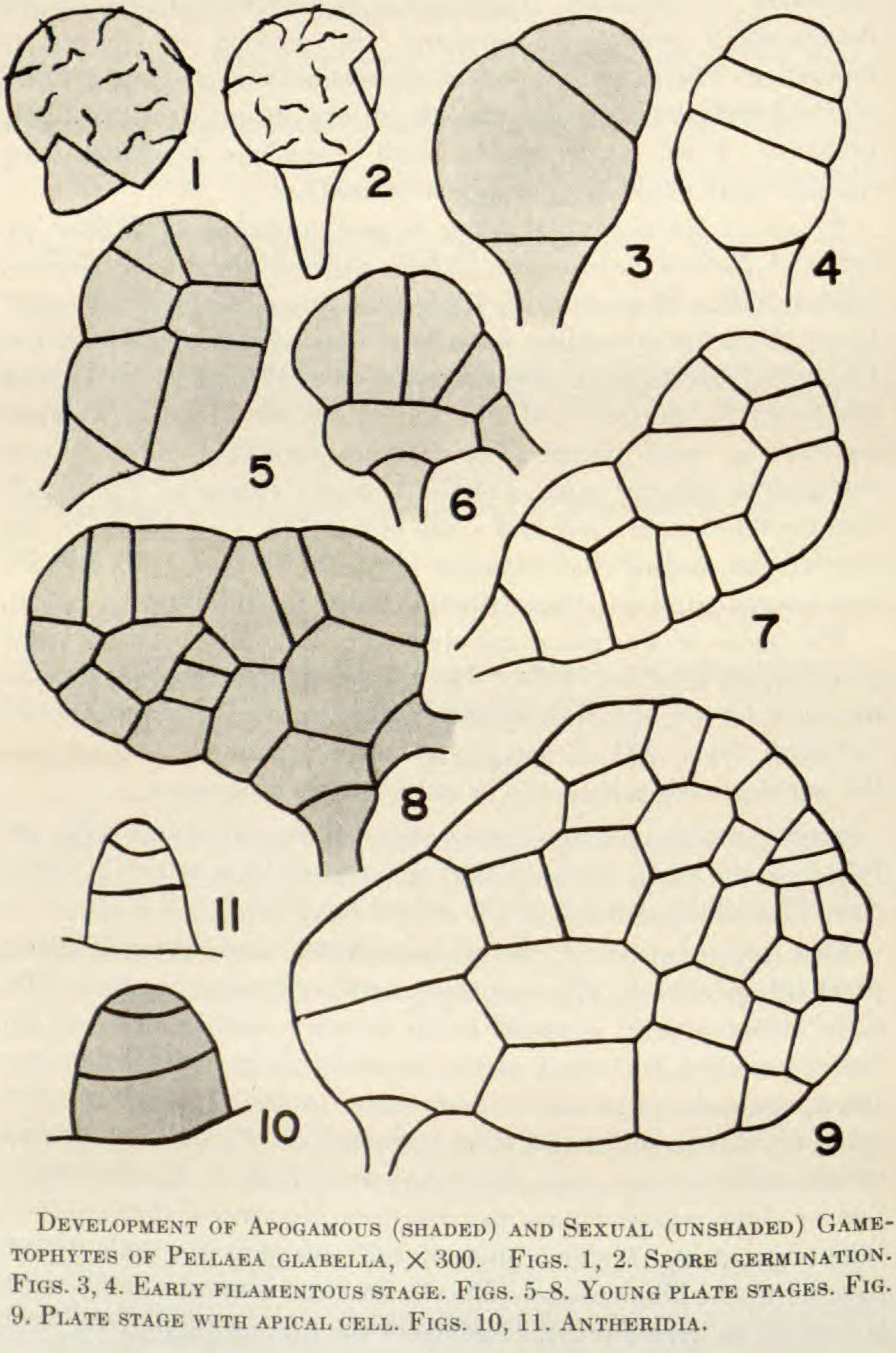
Spores of the sexual (*Whittier 3*) and apogamous (*Whittier 10*) forms of *Pellaea glabella* var. *glabella* were obtained from DeSoto, Missouri (See Wagner *et al*, 1965) and Rome, Tennessee, respectively. Voucher specimens have been deposited in the Vanderbilt University Herbarium. Aseptic techniques (Whittier, 1964) were employed to sterilize and sow the spores on 25 ml of nutrient medium in petri plates. The medium consisted of Knudson's solution of mineral salts, FeEDTA, minor elements, 1% or 0% sucrose, 0.6% agar, and had a pH of 6.0 (Whittier, 1964). In one experiment, antheridial hormone (Näf, 1958) at a 1/10 dilution was incorporated into the medium to insure antheridium formation.

The areas of the gametophytes were determined with a polar planimeter (Whittier, 1964). The measurements reported are the means of 64 individuals, and the standard errors of the means are included. Over 200 gametophytes were examined to determine the percent with archegonia or initial stages of apogamy.

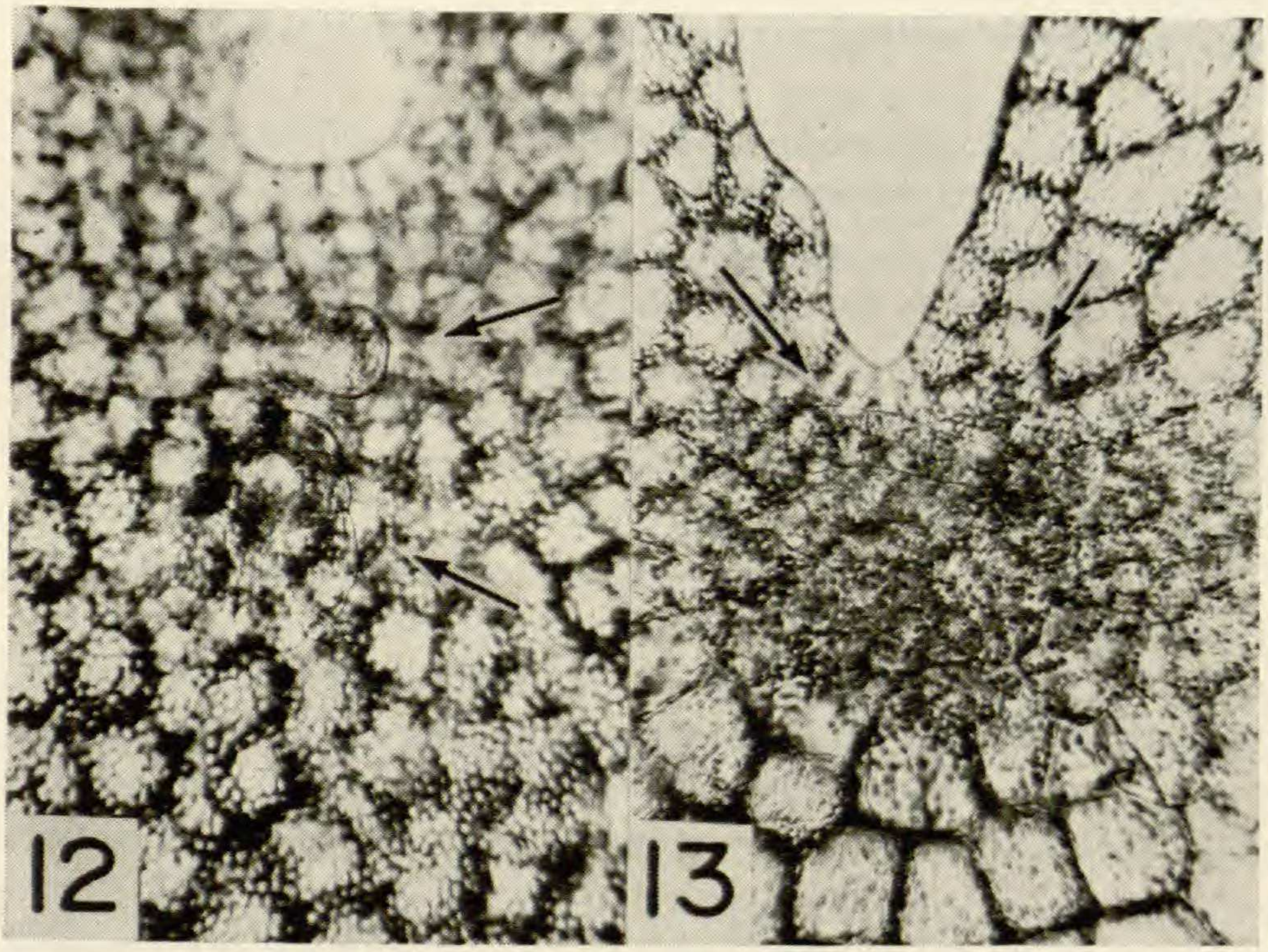
Spores of the apogamous and sexual forms are trilete with low ridges on the exine. Spores of the apogamous form exceed in diameter ($73.2 \pm 0.6\mu$) those of the sexual form ($55.7 \pm 0.5\ \mu$).

The development of the gametophytes was observed using prothalli grown on the nutrient medium lacking sucrose. The early development in both forms is very similar. The spores become swollen and crack at the triradiate ridge in three days for the apogamous form and in four days for the sexual form. The spore divides to produce the first prothallial cell and first rhizoid which enlarge to rupture the spore coat (*Figs. 1, 2*). Generally, two or three cell divisions give rise to a filament of three to four cells (*Figs. 3, 4*). A small plate of cells arises as the cells divide parallel to the filament axis (*Fig. 5*). In some instances no filament is formed, as irregular cell divisions of the first prothallial cell form



DEVELOPMENT OF APOGAMOUS (SHADED) AND SEXUAL (UNSHADED) GAMETOPHYTES OF PELLAEA GLABELLA, × 300. FIGS. 1, 2. SPORE GERMINATION. FIGS. 3, 4. EARLY FILAMENTOUS STAGE. FIGS. 5–8. YOUNG PLATE STAGES. FIG. 9. PLATE STAGE WITH APICAL CELL. FIGS. 10, 11. ANTHERIDIA.

a plate of cells directly (*Fig. 6*). With additional cell divisions and growth, larger plates develop (*Figs. 7, 8*) and ultimately an apical cell arises (*Fig. 9*). The plate continues to grow by the activity of the apical cell, but eventually a marginal meristem replaces the apical cell (*Fig. 12*) and a small cordate prothallus develops.



Apical Notch Region of Mature Cordate Gametophytes, × 200. Fig. 12. Sexual form showing marginal meristem and archegonia (arrows). Fig. 13. Apogamous form showing early apogamous development with sporophytic hairs (arrows).

In the presence of antheridial hormone, the young gametophytes form antheridia (*Figs. 10, 11*). The diameter of the antheridia are $48.6 \pm 0.4\,\mu$ and $36.2 \pm 0.3\,\mu$ for the apogamous and sexual forms respectively. No antheridia were observed unless antheridial hormone was added to the medium.

After the initiation of the small cordate prothallus, the development of the gametophytes differ. In the prothallus of the apogamous form, cells behind the notch divide to produce a mass of meristematic cells (*Fig. 13*) which develop into an apogamous sporophyte. The cells behind the notch in the sexual form give rise to a cushion with archegonia (*Fig. 12*). The archegonia and antheridia of the sexual form are functional because diploid juvenile sporophytes have been collected at DeSoto. Fertilization was not accomplished in culture, apparently due to technical difficulties.

TABLE I. SIZE COMPARISON (IN mm²) OF SEXUAL AND APOGAMOUS GAMETOPHYTES OF PELLAEA GLABELLA

	Sexual form		*Apogamous form*	
	0% sucrose	*1% sucrose*	*0% sucrose*	*1% sucrose*
Size on 21st day	0.20 ± 0.01	0.28 ± 0.01	0.39 ± 0.02	0.52 ± 0.02
Size at first apogamy or archegonia	1.31 ± 0.04	0.83 ± 0.08	1.18 ± 0.04	0.64 ± 0.02

The rate of prothallial development was compared between the two forms. The growth of the apogamous gametophytes is faster with or without sucrose (*Table I*) than is the growth of the sexual. In both forms prothallial growth is greater with sucrose in the nutrient medium than without it. The prothalli of the asexual form initiated apogamous sporophytes in a shorter period of time and on smaller prothalli than the gametophytes of the sexual form produced archegonia (*Table I*). On the 26th day after sowing the spores, 50% of the gametophytes of the asexual form on 0% sucrose had initiated apogamous sporophytes and none of the prothalli of the sexual form had produced archegonia. Five days later only 21% of the gametophytes of the sexual form had archegonia on 0% sucrose. With 1% sucrose, 79% of the gametophytes of the asexual form had apogamous developments and 21% of the gametophytes of the sexual form had archegonia on the 26th day. The presence of sucrose in the medium caused apogamous sporophytes and archegonia to be initiated sooner and on smaller gametophytes than without sugar (*Table I*). Thus gametophytes of the apogamous form were more efficient than those of the sexual form with or without sugar in the medium.

The findings of the present study on spore size and sculpturing confirm the reports of Wagner et al (1965) and Pickett and Manuel (1925). The growth of the gametophytes of the apogamous form in sterile culture is somewhat different from the growth on soil as reported by Pickett and Manuel (1925). The gametophytes in sterile culture have a shorter filamentous stage and the apogamous plant is initiated two months sooner than the gametophytes in soil culture. The development of the apogamous sporophyte from the gametophytic cells behind the apical notch appears to be the same under both conditions. Spore germination, gametophyte growth, and apogamous sporophyte initiation were faster in sterile culture than on the soil cultures of Pickett and Manuel.

The size differences between the spores and antheridia support the findings of Manton (1950) and Whittier (1966). Manton has reported that the diameter of spores and antheridia increases with an increase in the level of ploidy in *Osmunda.* Size differences between the apogamous and sexual form appear to be due to the difference in the chromosome number and not to the mode of sporophyte reproduction.

The promotion of gametophyte growth and the formation of apogamous plants on smaller prothalli earlier with sugar in the medium has been reported by Whittier (1964, 1965) in other fern genera. Since the initiation of archegonia was also accelerated by sugar, it appears that sugar similarly affects gametophyte development in both forms. The earlier conclusion (Whittier, 1964, 1965) that sugar brings about the conditions necessary for apogamy, and in this case archegonia, by affecting carbohydrate metabolism and the availability of respiratory substrate appears also true for *P. glabella* var. *glabella.*

In discussions of apogamy in ferns (Tryon & Britton, 1958; Stokey, 1948; Whittier, 1965) it has been noted that water for sperm movement is not required for apogamous reproduction. Gametophytes of an apogamous species can grow and produce sporophytes using only capillary water from the soil. Sporophytes cannot form by fertilization on gametophytes with mature sex organs with only capillary water (Duncan, 1941) because a film of water is necessary for sperm movement to the archegonium. Earlier investigators (Hayes, 1924; Stokey, 1948; Tryon & Britton, 1958) noted the advantage that apogamous species have in drier habitats because this additional water is unnecessary.

In *P. glabella* var. *glabella* apogamous reproduction has other advantages over sexual reproduction. The spores of the apogamous form germinate sooner and the prothalli grow faster than the sexual form. Of more importance is the initiation of the apogamous sporophyte before archegonia are initiated on the gametophytes of the sexual form. This initiation occurred on about 50% of the gametophytes before archegonia were formed on any of the gametophytes of the sexual form. Archegonia were formed shortly after the initiation of apogamous sporophytes but the presence of archegonia on the gametophytes of the sexual form is not equivalent to the initiation of apogamous plants on the gametophytes of the apogamous form. Conditions conducive for fertilization do not necessarily occur immediately after the formation of mature archegonia. Thus, besides the advantage of only requiring capillary water, apogamous reproduction occurs faster than sexual reproduction in *P. glabella* var. *glabella*. Since the gametophyte is more susceptible to desiccation than the sporophyte, more rapid sporophyte formation is important because less time in the life history of the plant is spent as a gametophyte. The conclusion that apogamous reproduction is more rapid and more advantageous than sexual reproduction in *P. glabella* var. *glabella* is supported by the greater range of the apogamous form.

An explanation of the early maturation of the $4x$ gametophytes of the apogamous form is impossible at this time because in *P. glabella* var. *glabella* two variables are involved. The gametophytes of the apogamous form have the $4x$ chromosome level and an asexual method of forming sporophytes while the gametophytes of the sexual form are haploid and produce sex organs. Whether the differences between the prothallial growth and development of the two forms is related to the variation in chromosome number or mode of sporophyte formation awaits further study.

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The Gametophyte and Juvenile Leaves of Loxogramme

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Some years ago I described spore germination and prothallus development in two Indian species of *Loxogramme*, based on materials collected in the field along with samples of the sporophyte (Nayar, 1955). The prothalli were collected on the tufted, sponge-like root masses of *L. involuta* and *L. lanceolata*. It was soon evident that reporting mature prothalli as cordate possibly was a mistake. Since then I have made several attempts to raise pure cultures of *Loxogramme* prothalli in the laboratory and to review the nature and development of these prothalli.